



## Article

# Gibberellic Acid Concentrations and Storage of *Caryocar brasiliense* (Caryocaraceae) Seeds Propagated in Tubes

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**Abstract:** The immersion of seeds in gibberellic acid solutions can promote dormancy breaking. Storage can be dynamic in seed longevity. The objective of this work was to evaluate different concentrations of gibberellic acid applied to seeds isolated from two storage times on the emergence of *C. brasiliense* tree seedlings grown in tubes. There were two experiments with different planting and storage times. The seeds were extracted from the endocarp. In both, a randomized block design was used with the following seven treatments: 0, 50, 100, 500, 1000 and 2000 mg L<sup>-1</sup> of GA<sub>3</sub> and dry seeds without pre-soaking, with five replications of 20 seeds. The seeds were treated with fungicide and sown in tubes containing substrate. After 60 days, evaluations and transplantation were carried out. In the experiment with older pyrenes (seeds stored for seven months), there was high seed mortality (96.54%) and a small emergence rate (3.45%). On the other hand, when new pyrenes were used, the percentage of dead seeds was 32.71% and emergence was 62.28%. GA<sub>3</sub> did not stimulate germination or the diameter of *C. brasiliense* tree seedlings, except for stem length. The 2000 concentration provided a greater stem length and greater leaf area compared to dry seeds.

**Keywords:** *Caryocar brasiliense* Cambess.; dormancy; germination; plant hormone; pequi; pequiizeiro



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## 1. Introduction

The *Caryocar brasiliense* Cambess. (“pequiizeiro”) is one of the most economically and socially important fruit species in the Brazilian Cerrado (Neotropical savanna) biome, since it generates income and employment for populations that live on extractivism in this biome [1]. In addition to the fruit pulp used in cooking, endosperm oil has desirable characteristics in the production of cosmetics, as well as its potential for use as biodiesel [2]. It is also an antioxidant and used in medicine to alleviate acute liver injury [3].

The exploitation of the *C. brasiliense* tree is performed almost exclusively through the intense extraction of its fruits, which places the survival of the species and its entire production chain at risk in the future, affecting the natural propagation and causing a decrease in native populations, as well as deforestation [4]. The commercial cultivation of the *C. brasiliense* tree is limited by the difficulty in obtaining quality seedlings, due to the dormancy of its seeds, which leads to low, slow, and uneven germination [5]. Dormancy is considered a factor of resistance to climatic variations [6].

Anatomical and reserve mobilization studies of *C. brasiliense* seeds have indicated that physiological dormancy is determined by the low potential for embryonic growth associated with the mechanical strength provided by the endocarp [7]. Recommended methods for overcoming this dormancy are the use, alone or together, of the plant regulator gibberellic acid (GA<sub>3</sub>) and the removal of the structures that surround the seed [8].

The use of GA<sub>3</sub> in *C. brasiliense* seeds increases the germination percentage and reduces the mean emergence time. In addition, it opens the possibility of standardizing and

improving the initial development of seedlings, which contributes significantly—in relation to germination under natural conditions—to enabling the commercial propagation of the species [9]. According to [10], this hypothesis is consistent with the fact that seed treatment with GAs in general can replace a positive signal in breaking dormancy.

The aerial sowing technology, using tubes and appropriate substrates, provides the production of quality seedlings on a large scale and at a lower cost, in addition to the tubes being reusable, occupying a smaller area, and being more easily transported [11]. This technology is more accessible to implement and is associated with methods of overcoming dormancy, the application of GA<sub>3</sub>, and the removal of seed cover, enabling it to contribute to developing a protocol that allows the supply of quality *C. brasiliense* seedlings to be increased.

However, it is necessary to define the concentrations of GA<sub>3</sub> that allow raising and standardizing germination, as well as maximizing seedling vigor. In addition, it is also important to evaluate the planting of seeds stored for different times, in order to expand the supply of seedlings during the year. The storage time can negatively or positively influence the viability, and consequently, the germination of *C. brasiliense* seeds. Therefore, it is necessary to accumulate studies to acquire a scientific basis that enables the creation of a germination protocol of *C. brasiliense* to finally be contemplated in the Seed Analysis Rules (RAS) of the government of Brazil and International Seed Testing Association (ISTA) [12].

The objective of this work was to evaluate concentrations of GA<sub>3</sub> applied to *C. brasiliense* seeds isolated from the endocarp, and from different storage times, on the emergence and vigor of seedlings grown in tubes, as well as their initial development after transplanting.

## 2. Materials and Methods

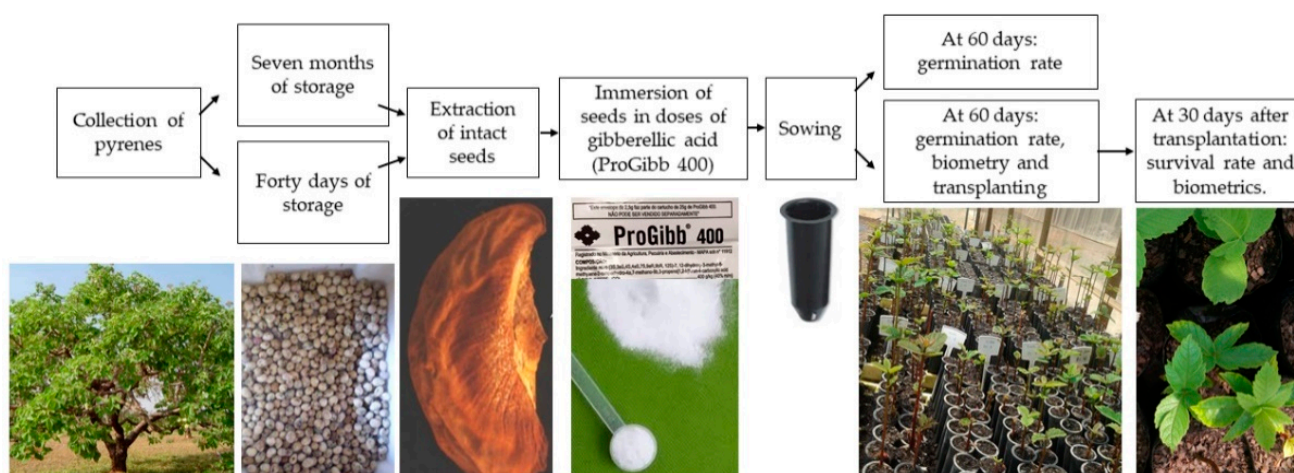
The pyrenes (seed + endocarp), freshly pulped by hand, with the use of a sharp knife (removal of the inner mesocarp), were collected in the municipality of São João da Lagoa, north of Minas Gerais, Brazil (16°54' S and 44°26' W), during the production season, spring, and summer. The pyrenes were transported in nylon bags to the Department of Plant Propagation of the Federal University of Minas Gerais—Campus Montes Claros, spread out in the shade at a temperature of 19 to 28 °C and relative humidity from 51 to 83%, in an open environment with good aeration to avoid the proliferation of fungi, for 30 days for drying; then, they were stored in nylon bags in a protected environment from wind, rain, and direct sunlight.

A scheme was made to summarize the steps of the experimental trial (Figure 1). Seed extraction was performed using an electric grinder to scarify the endocarp, a manual bench vise to press longitudinally the endocarp and provide a small opening, inverse pliers to open it further, and surgical forceps to remove the seed. Careful selection was carried out by eliminating empty seeds that had been attacked by pests and fungi and with the presence of some physical damage caused by the seed extraction process. The water content of the seeds was carried out by the oven method at 105 °C for 24 h [12].

Two experiments were set up equally but installed at different times and evaluated separately (the first with pyrenes stored for seven months; the second with pyrenes stored for 40 days). The *C. brasiliense* seeds were immersed for 24 h in the dark in different concentrations (0, 50, 100, 500, 1000, and 2000 mg L<sup>-1</sup>) of the plant regulator GA<sub>3</sub> (Progibb 400) and the control (additional treatment) with dry seeds (that is, not pre-soaked). After 24 h, the seeds that received the application of GA<sub>3</sub> and the dried ones were treated with the fungicide Vitavax-Thiram<sup>®</sup> 200 SC, from the chemical group Carboxanilide (Carboxin) and Dimethyldithiocarbamate (Tiram), with a concentration of 50% of the commercial product.

Sowing with the seeds was carried out vertically, with the plumule facing upwards and the radicle facing downwards, at a depth of 2 cm, in polyethylene tubes that were 12 cm high, 2.6 cm in internal diameter, and 3.3 cm in external diameter, with 6 grooves and a volume of 55 cm<sup>3</sup>, containing commercial substrate (Bioplant<sup>®</sup>). The tubes were placed in

a greenhouse with a temperature from 23 to 41 °C and relative humidity from 46 to 78% and irrigated with a manual irrigator twice a day to keep the substrate moist.



**Figure 1.** Scheme of the stages of carrying out the experimental test with the seeds of *C. brasiliense*.

For each experiment (from seeds stored for seven months and seeds stored for 40 days), the design adopted was in randomized blocks with the following 7 treatments: 0, 50, 100, 500, 1000, and 2000 mg L<sup>-1</sup> of GA<sub>3</sub> plus the control (dry seeds without pre-soaking), with 5 replications of 20 seeds each, 100 seeds per treatment, and 700 intact seeds for each experiment.

The germinated seedlings were counted to calculate the germination speed index (GSI), adding the number of seeds germinated per day and dividing it by the number of days after sowing [13]. At 60 days after sowing, in both experiments, the percentage of emergence and dead seeds (seeds spoiled, without consistency and with a bad odor) were measured. Seeds that did not germinate and did not die were considered dormant. Along with these evaluations, the biometry of the seedlings was performed, only from the experiment with pyrenes stored for 40 days, measuring the stem length (cm), stem diameter (cm), number of leaves, and leaf area (cm<sup>2</sup>). The length was determined with the aid of a ruler and the diameter by means of a digital caliper, while the leaf area was determined according to the methodology of [14].

Moreover, at 60 days after sowing, the germinated seedlings were transplanted into polyethylene plastic bags suitable for seedling production (size 10 × 18 cm and 0.18 mm thick), with substrate collected in a region with a high endemicity of native *C. brasiliense* plants mixed with manure in the proportion 3 (soil):1 (manure). They were then transferred from the greenhouse to the pond with 50% shade, temperature from 17 to 28 °C, and relative humidity from 64 to 88%, and irrigated by inverted micro sprinkler twice a day, keeping the substrate moist, without waterlogging. After 30 days from the transplanting date, the survival rate was determined, relating live and dead seedlings, as well as a new seedling biometry identical to that performed at 60 days post-planting, but independent of it, since the seedlings were in other conditions of pond, container, and substrate.

The percentage data were transformed to  $y = \arcsin (\times / 100)^{0.5}$ , then submitted to an analysis of variance (ANOVA), and the means were compared by the Tukey test at  $p < 0.05$  using the Sisvar software version 5.6 (Lavras, MG, Brazil). The graphical results were presented as mean value ± standard deviation (n = 3). For the parameters of the experiment with the seeds stored for 40 days, a principal component analysis (PCA) was performed using the statistical software JMP 10 (SAS Institute Inc., Cary, NC, USA).

### 3. Results and Discussion

#### 3.1. Seeds Stored for Seven Months

For seeds stored for seven months, there was a low percentage of germination in all treatments, but GA<sub>3</sub> stimulated germination in isolated *C. brasiliense* seeds, mainly at concentrations of 100 and 500 mg L<sup>-1</sup>, which differed from concentrations of zero and 2000 mg L<sup>-1</sup> (Table 1). In the other variables evaluated (germination speed index (GSI) and dead seeds), there was no significant difference. The GSI was low, and the percentage of dead seeds was high, consequently resulting in few seedlings, making it impossible to obtain the seedling growth rate. It should be noted that there was still seed loss due to fungal attack, even with fungicide treatment and phytosanitary care.

**Table 1.** Germination speed index (GSI), percentage of germination and dead seeds of *C. brasiliense* stored for seven months, pre-soaked in concentrations of GA<sub>3</sub> and without pre-soaking (dry seeds) at 60 days after sowing in tubes.

| GA <sub>3</sub> (mg L <sup>-1</sup> ) | GSI     | Germination (%) | Dead Seeds (%) |
|---------------------------------------|---------|-----------------|----------------|
| Dry seeds                             | 0.01 a* | 2.71 abc        | 97.29 a        |
| 0                                     | 0.01 a  | 0.84 c          | 99.16 a        |
| 50                                    | 0.02 a  | 4.17 ab         | 95.83 a        |
| 100                                   | 0.03 a  | 6.04 a          | 93.96 a        |
| 500                                   | 0.02 a  | 6.25 a          | 93.75 a        |
| 1000                                  | 0.01 a  | 2.50 abc        | 97.50 a        |
| 2000                                  | 0.01 a  | 1.67 bc         | 98.33 a        |
| Overall average                       | 0.01    | 3.45            | 96.54          |
| CV (%)                                | 1.17    | 26.95           | 3.73           |
| MSD                                   | 0.01    | 0.97            | 6.44           |

\* Means followed by the same letter in the column do not differ statistically from each other by the Tukey test at  $p < 0.05$  and means followed by different letters in the column differ statistically from each other by the same test. CV, coefficient of variation; MSD, minimum significant difference.

The dry seeds just extracted from the pyrenes and stored for seven months presented 6.17% of water content. This observed content is close to that found by [9] in stored *C. brasiliense* seeds. According to these authors, the freshly dispersed seed has a water content close to 40% and reduces after a little less than a month to 7%. The low germination rate and high seed mortality is associated with the vigor and integrity of seed membranes, since *C. brasiliense* seeds decrease their vigor over time [9]. It is important to highlight that the *C. brasiliense* seed has high lipid reserves, in which they are subject to oxidative processes [7].

The oxidative process occurs naturally in seeds, where aging results in weakening and the loss of membrane integrity and vigor [15,16]. Biochemical changes such as the oxidation of lipid reserves, which are abundant in *C. brasiliense* seeds, promote irreparable damage to cells, membranes, proteins, carbohydrates, and DNA, which leads to seed deterioration [15–17]. In addition, the high rate of water absorption by the isolated seed can accentuate damage to the membrane, whose integrity is reduced, leading to the death of the embryo's tissues.

#### 3.2. Seeds Stored for 40 Days

The dry seeds just extracted from the pyrenes and stored for 40 days had a water content of 6.35%. There was a significant difference for the percentage of germination and dead seeds, especially a concentration of 100 mg L<sup>-1</sup>, which was lower and higher than the zero concentration, respectively, for the percentage of germination and dead seeds. Due to the introduction of such an amount of GA<sub>3</sub> into the cells, unlike the zero concentration in the natural state, it could probably have caused a physiological imbalance in the ratio between GA<sub>3</sub> and abscisic acid. No significant difference was observed for GSI. The average percentages of germination of the experimental test (62.28%) and GSI (0.68) were much higher compared to seeds stored for seven months, in addition to the average percentage

of dead seeds (32.71%) being much lower in seeds stored for 40 days (Table 2). Seeds not germinated but with good consistency and color were considered dormant seeds.

**Table 2.** Germination speed index (GSI), percentage of germination and dead seeds, number of leaves, leaf area and stem diameter in *C. brasiliense* seedlings from seeds stored for 40 days, pre-soaked in concentrations of GA<sub>3</sub> and dried, without pre-imbibition, at 60 days after sowing in tubes.

| GA <sub>3</sub><br>(mg L <sup>-1</sup> ) | GSI     | Germination<br>(%) | Dead<br>Seeds (%) | Number<br>of Leaves | Leaf Area<br>(cm <sup>2</sup> ) | Stem<br>Diameter<br>(cm) |
|--|---------|--------------------|-------------------|---------------------|---------------------------------|--------------------------|
| Dry seeds                                | 0.59 a* | 67.00 ab           | 23.00 cd          | 3.09 b              | 8.03 b                          | 3.02 a                   |
| 0  | 0.73 a  | 74.00 a            | 20.00 d           | 4.17 b              | 9.90 ab                         | 3.10 a                   |
| 50                                       | 0.72 a  | 64.00 ab           | 27.00 bcd         | 4.55 a              | 11.20 ab                        | 3.10 a                   |
| 100                                      | 0.55 a  | 47.00 b            | 52.00 a           | 4.52 a              | 9.54 ab                         | 3.19 a                   |
| 500                                      | 0.82 a  | 69.00 ab           | 28.00 bcd         | 4.80 a              | 11.08 ab                        | 3.36 a                   |
| 1000                                     | 0.75 a  | 60.00 ab           | 38.00 abc         | 4.70 a              | 11.21 ab                        | 3.02 a                   |
| 2000                                     | 0.61 a  | 55.00 ab           | 41.00 ab          | 4.50 a              | 12.34 a                         | 2.93 a                   |
| Overall<br>average                       | 0.68    | 62.28              | 32.71             | 4.33                | 10.35                           | 3.10                     |
| CV (%)                                   | 19.06   | 10.29              | 10.92             | 14.02               | 14.83                           | 11.51                    |
| MSD                                      | 0.03    | 1.64               | 1.26              | 1.50                | 3.84                            | 0.56                     |

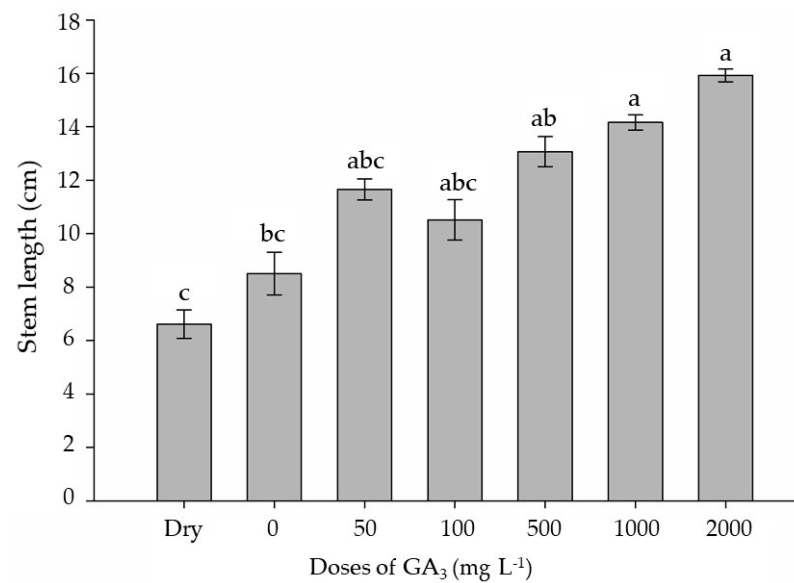
\* Means followed by the same letter in the column do not differ statistically from each other by the Tukey test at  $p < 0.05$  and means followed by different letters in the column differ statistically from each other by the same test. CV, coefficient of variation; MSD, minimum significant difference.

In contrast to what was found in other studies [5,9,18], in this experimental assay, GA<sub>3</sub> did not promote increases in germination indices, even in seeds stored for 40 days. *C. brasiliense* seeds show physiological dormancy [7,9]. Plant hormones, such as gibberellins and abscisic acid, control germination by acting as the substances responsible for activating or inactivating enzymes that maintain or not the embryo in the dormant state [8,19].

A similar result was obtained by [14] when evaluating the germination of *C. brasiliense* seeds under storage periods; a reduction was observed in the germination percentage from 70% (fresh seeds) to 19% after four months of storage. The same author also observed a high rate of fungal infestation in the stored seeds. Although GA<sub>3</sub> has a proven effect on seed dormancy, the effect can be variable depending on genetic material, quality, age, origin, and the intensity of seed dormancy [8,19]. This fact also explains what happened in the study by [20], who, in the field, did not obtain significance in the germination of *C. brasiliense* tree seeds with endocarp treated with GA<sub>3</sub> 500 mg L<sup>-1</sup>, fungicide (Tecto®), and insecticide (Tuity®).

Seed vigor interferes with germination capacity, seedling establishment, germination speed index, and seed deterioration [16]. In this way, seeds extracted from recently collected pyrenes or with storage for less than 40 days may have a higher germination rate. Thus, it was observed that seeds stored for 40 days supported pre-imbibition treatments better, since the “new” seed had a lower mortality rate. Therefore, like what was found by [9], while storage may partially overcome the dormancy in *C. brasiliense* seed stored for 20 days, it also reduces its vigor over time.

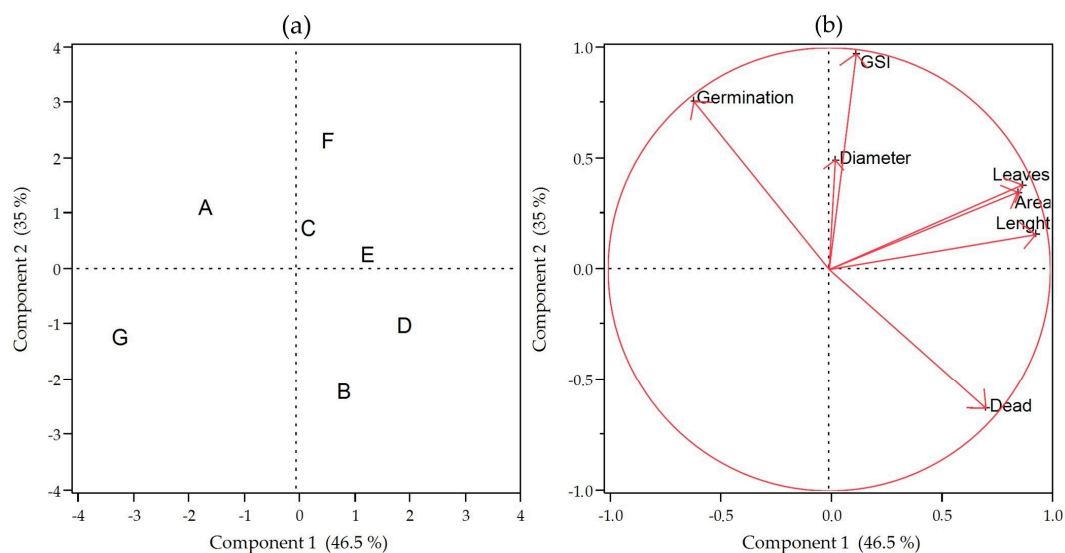
In the first biometric evaluation, 60 days after sowing, an effect of GA<sub>3</sub> on leaf emission was observed, as shown in Table 2, where the number of leaves was higher in those seedlings from seeds immersed in GA<sub>3</sub>. In the leaf area, seedlings from seeds immersed in 2000 mg L<sup>-1</sup> of GA<sub>3</sub> had a significant effect, surpassing only dry seeds (Table 2). The 1000 and 2000 mg L<sup>-1</sup> concentrations of GA<sub>3</sub> in the seeds did not influence the seedlings' stem diameter, however, it provided an increase in the stem length (Figure 2), while the dry seeds and zero concentration were significantly lower.



**Figure 2.** Stem length (cm) of *C. Brasiliense* tree seedlings from seeds stored for 40 days, pre-soaked in concentrations of GA<sub>3</sub> (mg L<sup>-1</sup>) and dry seeds, without pre-imbibition, at 60 days after sowing in tubes. Equal letters represent equal averages and different letters represent different averages by the Tukey test at  $p < 0.05$ .

### 3.3. Analysis of Principal Components of the Parameters Evaluated in Seeds Stored for 40 Days

The principal component analysis (PCA) of seven parameters evaluated in *C. brasiliense* tree seedlings in seven treatments (six concentrations of GA<sub>3</sub> and dry seeds) allowed the general observation of data separately regarding treatments in different quadrants (Figure 3). Total variability was explained by three PCs of eigenvalues > 1.0. However, two of them were considered the most important, as they had eigenvalues > 2.0. Of these three PCs, the first two (PC1 and PC2) represented 81.5% of the total variation.



**Figure 3.** Score graph (a) and load graph (b) of PCA of seven parameters evaluated in *C. brasiliense* seedlings at 60 days after sowing, from seeds stored for 40 days, submitted to seven treatments (six concentrations of GA<sub>3</sub> and dry seeds without pre-soaking). Score chart legend (a): 0 mg L<sup>-1</sup> of GA<sub>3</sub> (A), 50 (C), 100 (B), 500 (F), 1000 (E), 2000 (D), and dry seeds (G). Load graph legend (b): stem length, stem diameter, number of leaves, leaf area, germination speed index (GSI), percentage of germination, and percentage of dead seeds.

PC1 was responsible for 46.5% of the total variation, being effective in separating treatments with the absence of GA<sub>3</sub> (zero concentration and dry seeds) in negative scores, from treatments with the presence of GA<sub>3</sub>, 50, 100, 500, 1000, and 2000 mg L<sup>-1</sup> of GA<sub>3</sub>, in positive scores (Figure 3a). The analysis of PC1 loads (Figure 3b) suggests that this separation is due to the percentage of germination that is in negative load and to the parameters stem length, number of leaves, leaf area and percentage of mortality, since all these characteristics presented positive charges (>0.70) on the PCA. PC1 scores and loads showed that GA<sub>3</sub>, although it did not promote germination, provided a greater number of leaves. The 2000 mg L<sup>-1</sup> concentration provided a greater leaf area compared to the seedlings originating from dry seeds, and together with the 1000 mg L<sup>-1</sup> concentration it provided greater stem length.

According to the PCA, in the biometric evaluations of the seedlings there were positive correlations between the stem length, number of leaves, and leaf area. Thus, it was evidenced that these analyses behaved in a similar way, with the same tendency to increase the observed values. For the evaluations of seed germinability, there was a positive correlation between the GSI and the percentage of germination and a negative correlation between the percentage of mortality with the percentage of germination and GSI. With the increase in germination, the GSI also increased, and seed mortality decreased.

PC2 represented 35% of the total variation; this was mainly related to GSI and the percentage of germination with positive loads, and the percentage of dead seeds with negative loads. PC2 in the score chart was important to separate treatments of zero, 50, 500, and 1000 mg L<sup>-1</sup> of GA<sub>3</sub> from the others, since these treatments had positive scores. The 100 mg L<sup>-1</sup> concentration of GA<sub>3</sub> presented the most evident negative score, in the same way that the percentage of dead seeds presented the most expressive negative load, corroborating the mean test in Table 2. Therefore, the analysis of the PCs was efficient in confirming the results presented here.

### 3.4. Parameters Evaluated after Transplanting to Bags

The developmental characteristics of the 316 (40 to 50 seedlings for each concentration) seedlings were evaluated at 30 days after transplanting the seedlings from seeds stored for 40 days into bags (Table 3). Stem diameter, number of leaves, and leaf area did not show significant differences in response to GA<sub>3</sub> concentrations, whereas stem length was greater in 2000 mg L<sup>-1</sup> compared to the dry seeds.

**Table 3.** Number of leaves, leaf area, stem diameter, stem length, and survival rate of *C. brasiliense* tree seedlings from seeds stored for 40 days, pre-soaked in concentrations of GA<sub>3</sub> and dry seeds, without pre-soaking, at 30 days after transplanting into plastic bags.

| GA <sub>3</sub><br>(mg L <sup>-1</sup> ) | Number of<br>Leaves | Leaf Area<br>(cm <sup>2</sup> ) | Stem<br>Diameter<br>(cm) | Stem Length<br>(cm) | Survival<br>Rate (%) |
|--|---------------------|---------------------------------|--------------------------|---------------------|----------------------|
| Dry seeds                                | 3.40 a*             | 10.40 a                         | 3.60 a                   | 11.20 b             | 78.57 a              |
| 0  | 3.80 a              | 10.40 a                         | 4.00 a                   | 14.40 ab            | 62.50 a              |
| 50                                       | 3.80 a              | 12.40 a                         | 3.80 a                   | 15.60 ab            | 79.10 a              |
| 100                                      | 4.20 a              | 12.40 a                         | 3.80 a                   | 16.40 ab            | 91.07 a              |
| 500                                      | 3.80 a              | 11.60 a                         | 3.60 a                   | 16.20 ab            | 72.30 a              |
| 1000                                     | 3.40 a              | 10.20 a                         | 3.60 a                   | 17.60 ab            | 63.15 a              |
| 2000                                     | 3.40 a              | 12.80 a                         | 3.40 a                   | 18.40 a             | 63.04 a              |
| CV (%)                                   | 21.27               | 25.00                           | 5.14                     | 12.26               | 18.01                |
| MSD                                      | 0.08                | 0.17                            | 0.01                     | 0.09                | 0.32                 |

\* Means followed by the same letter in the column do not differ statistically from each other by the Tukey test at  $p < 0.05$  and means followed by different letters in the column differ statistically from each other by the same test. CV, coefficient of variation; MSD, minimum significant difference.

The immersion of seeds at increasing concentrations of GA<sub>3</sub> presents a linear behavior in stem length (Figure 2), influencing the initial growth of seedlings, after transplanting; although no linear behavior was observed, a trend was observed, since seedlings from seeds that received the highest concentration of GA<sub>3</sub> were superior to dry seeds (Table 3). Regarding the survival rate, no significant differences were observed, with an average of 72.81%.

The exogenous application of GA<sub>3</sub> causes an excessive elongation/growth of internodes in the stems, as already observed in *C. brasiliense* tree seedlings [21]. This growth is promoted through cell elongation due to the loosening of the cell wall, reducing its resistance, generating an increase in cell turgor, and causing cell expansion [22,23].

The expression of genes that promote cell elongation and division are regulated by GAs, which induce the expression of expansins and endoglyoxyglucan, causing changes in the cell wall that allow the turgor-driven expansion of cells [22,24]. Gibberellins stimulate the production of hydrolytic enzymes in germinating seeds, with the production and secretion of enzymes such as  $\alpha$ -amylase in the aleurone layer, for the breakdown of macromolecules in the endosperm, which is a source of nutrients for the growing embryo and contributes to the rapid establishment of seedlings [23,24].

Physiological studies and the phenotypic characterization of mutants deficient in gibberellin biosynthesis revealed that GAs play an important role in internode elongation, since these mutants showed signaling and expression of genes that promote cell expansion and division when treated with GA, and consequently, tissue growth [22–24]. In a similar way to the one presented, using gibberellin, an increase in stem elongation is observed in dwarf plants, rosettes, genetic dwarf peas (*Pisum sativum* L.), dwarf corn (*Zea mays* L.), and many other plants, as GA<sub>3</sub> promotes cell elongation and drives internode elongation in seedlings [10].

Gibberellins also control the expression of genes related to auxin biosynthesis, which is known to stimulate cell expansion [24]. Thus, this feat cannot be attributed to an isolated factor, since auxins and gibberellins are related. A clear example is the necessity of gibberellin biosynthesis for the transport of auxins, which promotes stem elongation [23,24].

The effect of GA<sub>3</sub> on germination, elongation, and cell division is quite clear [8,19,23], which explains the stem growth observed in this research. The same does not occur in the stem diameter, since the process promotes rapid internode elongation and vertical growth. Further, the biosynthesis of compounds such as gibberellins and auxins is carried out in the meristems, which are more active regions; although translocated in the phloem, this favors growth in both directions, either in stem length or root length [10,24].

It is observed that the elongation and cell division caused by GA<sub>3</sub> not only influenced the growth of the stem, but was also responsible for the emission of leaves and leaf area, although in a discrete way. According to [10], leaf area is important in the accumulation of dry mass in plants, since larger leaf areas result in larger surfaces, and consequently, a greater production of photoassimilates.

Concentration 100 (mg L<sup>-1</sup> of GA<sub>3</sub>) expressed the highest mean survival rate but did not significantly differ from the others, although it expressed the highest percentage of dead seeds in the germination test. The non-survival of some seedlings was probably due to low winter temperatures, with a minimum of 13 °C (INMET), which reduces sap conduction associated with transplanting stress and the sensitivity of the *C. brasiliense* root system. Tropical native tree species subjected to climate variables with low temperatures decrease the number of leaves and shoots [10,25].

The tubes (51 cm<sup>3</sup>) of the present study proved to be useful for the propagation of the *C. brasiliense* tree. [26]; evaluating the production of seedlings of the forest species *Cabralea canjerana* (Vell.) in tubes of 100 and 280 cm<sup>3</sup> did not show a significant difference between the recipients for shoot dry mass, total dry mass, and Dickson quality index. To confirm the differences in the production methods of *C. brasiliense* tree seedlings in the nursery, post-planting evaluations in the field are necessary to verify such behavior as a function of the recipient, stored seeds, and GA<sub>3</sub>.

#### 4. Conclusions

GA<sub>3</sub> applied to *C. brasiliense* seeds does not stimulate seedling emergence in both storage times (40 days and seven months). Seeds from pyrenes stored for seven months have a high mortality after two months of sowing and a low percentage of germination, whereas seeds stored for 40 days have a lower mortality rate and a higher germination rate, so newer seeds are indicated for propagation of this species. It is important to pre-soak the seeds before sowing them. The concentration of 2000 mg L<sup>-1</sup> of GA<sub>3</sub> favors a greater development of the aerial part of *C. brasiliense* tree seedlings cultivated in tubes. The use of tubes is viable for the propagation of the *C. brasiliense* tree.

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